Hemoglobin Binding of Arylamines and Nitroarenes: Molecular Dosimetry and Quantitative Structure–Activity Relationships

Gabriele Sabbioni

Institute of Pharmacology and Toxicology, University of Würzburg, Würzburg, Germany

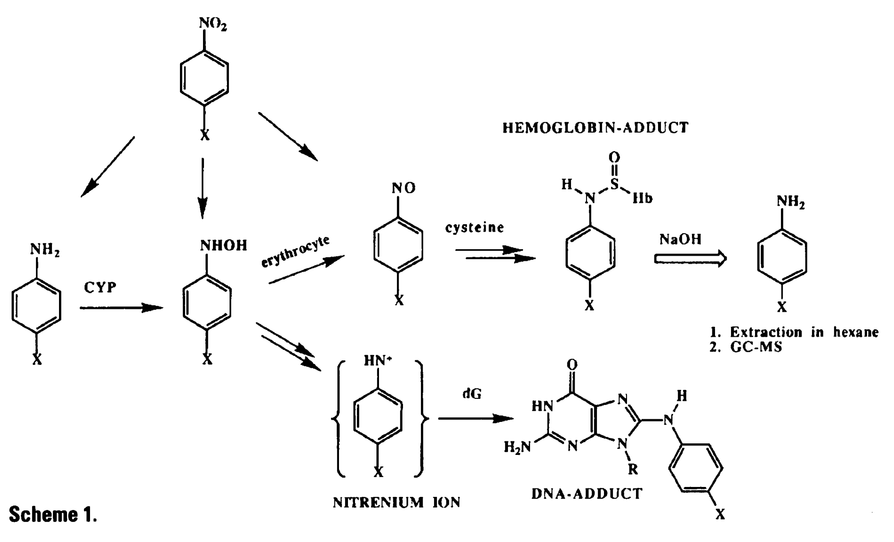
N-Oxidation and nitroreduction to yield *N*-hydroxyarylamines are metabolic steps that are crucial for the genotoxic properties of aromatic amines and nitroarenes, respectively. *N*-Hydroxyarylamines can form adducts with DNA, tissue proteins, and the blood proteins albumin and hemoglobin in a dose-dependent manner. The determination of hemoglobin adducts is a useful tool for biomonitoring exposed populations. We have established the hemoglobin binding index (HBI) [(mmole compound/mole Hb)/(mmole compound/kg body weight)] of several aromatic amines and nitroarenes in female Wistar rats. Incorporating values obtained by other researchers in the same rat strain, the logarithm of hemoglobin binding (log HBI) was plotted against several physicochemical parameters and against calculated electronic descriptors of nitroarenes and arylamines. Most arylamines and nitroarenes form hydrolyzable (e.g., sulfinamide) adducts with hemoglobin in rats. The amount of hemoglobin binding decreases with the oxidizability of the arylamines, except for compounds that are substituted with halogens in *ortho* or *meta* position. For halogen-substituted arylamines, the amount of hemoglobin binding is directly proportional to the pK_a. Hemoglobin binding of nitroarenes increases with the reducibility of the nitro group. The structure activity relationships (SAR) for hemoglobin binding of nitroarenes and arylamines are comparable. The SAR found for hemoglobin binding were compared with the SAR found in the literature for mutagenicity, carcinogenicity, and cytotoxicity of arylamines and nitroarenes. In general, the mutagenicity or carcinogenicity of arylamines increases with their oxidizability. This first set of data suggests that the levels of hemoglobin binding, mutagenicity, and carcinogenicity of arylamines are not determined by the same electronic properties of the compounds, or not by these properties alone. These results indicate that hemoglobin binding may prove not to be a useful index of the genotoxic potency of arylamines and nitroarenes. Further work will be performed to investigate whether high hemoglobin binding correlates with high cytotoxic potency of these compounds. — Environ Health Perspect 102(Suppl 6):61–67 (1994)

Key words: hemoglobin adducts, molecular dosimetry, aromatic amines, nitroarenes, QSAR

Introduction

Aromatic amines and nitroarenes are very important intermediates in industrial manufacturing of dyes, pesticides, and plastics and are significant environmental pollutants. Ring oxidation, *N*-glucuronidation, *N*-acetylation, and *N*-oxidation are the major metabolic pathways of arylamines in mammals [review of metabolism in (*1,2*)]. *N*-Oxidation is a crucial step in the metabolism of arylamines and aromatic amides to toxic products. Arylamines are metabolized in the liver by monooxygenases to highly reactive *N*-hydroxyarylamines. Nitroarenes are reduced by microorganisms in the gut or by nitroreductases and aldehyde dehydrogenase in hepatocytes to nitrosoarenes and *N*-hydroxyarylamines (*3*). *N*-Hydroxyarylamines can be further metabolized to *N*-sulfonyloxyarylamines, *N*-acetoxyarylamines or *N*-hydroxyarylamine *N*-glucuronide. These highly reactive intermediates are responsible for the genotoxic and cytotoxic effects (*4–7*) of this class of compounds. They react with DNA and proteins (Scheme 1). For 4-aminobiphenyl (4ABP) (*8*), it has been shown that the same metabolite—the *N*-hydroxyarylamine—reacts with DNA of the bladder and with hemoglobin.

The main goal of the present work was to determine the bioavailability of the *N*-hydroxyarylamine for an array of substituted arylamines and nitroarenes by measuring the amount of hydrolyzable adducts formed with rat hemoglobin. The relationships between hemoglobin binding of nitroarenes and arylamines and their



Scheme 1.

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Address correspondence to Dr. Gabriele Sabbioni, Institute of Pharmacology and Toxicology, University of Würzburg, Versbacher Str. 9, D-97078 Würzburg, Germany. Telephone 49 0931 201 2281. Fax 49 0931 201 3446.

Table 1. Heat of formation of arylamines and their corresponding nitrenium ions calculated by MNDO, AM1, and PM3.[a]

Amine	MNDO amine	MNDO nitrenium	AM1 amine	AM1 nitrenium	PM3 amine	PM3 nitrenium	$E^1/_2$ [V]	HBI
4MSA	14.7	226.7	19.6	224.8	21.0	232.8	0.50	3.8
A	21.7	241.5	20.5	246.9	21.3	244.9	0.71	22.0[b]
4FA	–24.7	197.5	–24.6	201.1	–22.3	203.1	0.67	33.0
2CA	15.1	239.6[c]	13.1	240.5[c]	14.7	233.6	0.76	0.5
3CA	14.0	240.8[c]	13.3	245.0[c]	14.4	241.4[c]	0.77	12.5
4CA	13.8	239.1	13.1	239.9	14.4	233.8	0.73	569.0[b]
4BrA	24.1	248.6	24.6	254.8	28.7	254.8	0.74	341.0
4IA	32.7	259.3	35.9	267.3	42.5	262.2	0.74	296.0
24DCA	8.0	237.4[c]	6.3	234.0[c]	8.1	224.4	0.80[d]	0.6
26DCA	9.2	238.6	6.3	235.2	8.3	227.4	0.84[d]	—
34DCA	9.0	239.9[c]	7.5	238.7[c]	9.2	230.7[c]	0.77	9.0[b]
35DCA	7.1	240.7	6.7	244.0	7.8	238.3	0.84	0.6
2MA	16.2	232.7	13.2	234.0	13.0	232.2	0.70[e]	4.0[b]
3MA	14.2	233.1[c]	13.0	237.7[c]	12.1	234.0[c]	0.71[e]	4.9[b]
4MA	13.8	231.0	13.0	232.6	12.0	229.7	0.62[e]	4.3[b]
2EA	12.3	227.7	8.0	227.7	8.5	227.9	0.67	5.1
3EA	9.4	227.9[c]	7.3	231.5[c]	7.4	229.1	0.69	12.7
4EA	9.0	225.5	7.1	226.2	7.3	225.7	0.64	5.8
24DMA	8.4	222.4	5.7	220.6	3.8	217.6	0.59[e]	2.3[b]
25DMA	8.6	224.2	5.7	224.8	3.7	221.3	0.65[d]	7.3
26DMA	11.6	225.1	6.0	222.6	5.0	220.6	0.63	1.1
34DMA	8.9	225.4[c]	6.1	224.5[c]	3.4	220.0[c]	0.59	0.7
35DMA	6.7	225.0	5.6	228.9	2.7	223.5	0.68[d]	14.0
245TMA	3.3	216.7	–1.2	212.4	–4.6	208.0	0.56[e]	0.7[b]
246TMA	3.9	215.3	–1.5	209.5	–4.2	206.3	0.53	0.2
3CNA	52.8	281.6[c]	52.1	288.0[c]	56.3	289.5[c]		1.5
3TFA	–126.7	106.5	–135.7	102.3	–137.1	97.5	0.84	28.4
4TFA	–128.2	111.7	–137.1	105.8	–138.0	100.8	0.89	148.0
24DFA	–69.6	155.5	–69.3	157.5[c]	–64.8	163.1[c]	0.74	32.0
3C4FA	–29.7	198.8[c]	–29.3	201.2[c]	–27.8	200.3[c]	0.78	30.7
2C4MA	7.3	229.0[c]	5.6	226.6[c]	5.4	219.9	0.68[e]	1.0
4C2MA	8.3	230.1[f]	5.8	227.5[f]	6.2	221.9[f]		28.0[b]
5C2MA	8.5	231.5[f]	6.0	231.7[f]	6.1	228.4[f]	0.75[e]	28.0[b]
6C2MA	9.9	230.8[f]	5.9	228.3[f]	6.4	222.3[g]	0.74[e]	0.8[b]
4ABP	46.4	257.3	45.9	259.7	45.3	256.1	0.60[e]	344.0[b]

[a]All heat of formation values are expressed as kcal/mole. [b]These data were obtained from Neumann (*39*). [c]Syn-conformer. [d]These values have been redetermined and revised from Sabbioni (*10*). [e]New experimental data. [f]The most stable conformer is with the methyl group *anti* to the proton on the nitrogen. [g]The most stable conformer is with the methyl group *syn* to the proton on the nitrogen.

electronic properties were studied. The structure-activity relationships (SAR) for hemoglobin binding were compared with those for their mutagenic and carcinogenic potency in order to test to which extent hemoglobin binding may be used as a predictor for genotoxicity.

Materials and Methods

The experimental details of the work with nitroarenes have been published elsewhere (*9*). The methods for the animal experiments, the isolation of hemoglobin, and the quantification of the arylamines bound to hemoglobin have been published recently (*10*). The aromatic amines and nitroarenes were given to female Wistar rats by gavage, and the rats were sacrificed 24 hr later. The hemoglobin was precipitated with ethanol, hydrolyzed in 0.1 M NaOH in the presence of recovery standards [e.g., 4-chloroaniline (4CA), d5-aniline] and extracted with hexane. The hexane fraction was analyzed by gas chromatography-mass spectrometry (GC-MS) with electron impact ionization in the single ion mode. Structure identification was based on the retention time and on the mass spectrum or the ratio of the main mass fragments. Arylamines with low hemoglobin binding were derivatized with pentafluoropropionic acid anhydride and analyzed by GC-MS. To establish whether the aromatic amines recovered from the alkaline hydrolysis were covalently bound, all samples were extracted with hexane at neutral pH and analyzed by GC-MS.

The electronic properties of the arylamines and the nitroarenes were calculated using the programs Modified Neglect of Differential Overlap (MNDO), Austin Model 1 (AM1), and Parametric Method number 3 (PM3), which are part of MOPAC 6.0 (Quantum Chemistry Program Exchange, Indiana University, Bloomington, IN) (*11*). The oxidizability of arylamines was determined experimentally by HPLC equipped with an electrochemical detector (*10*). The electrode potential was decreased stepwise (0.05 V) from 1 to 0.4 V. The peak integrals obtained (average of two injections) were plotted against the electrode potential. The half wave oxidation potential ($E_{1/2}$) was obtained from the resulting hydrodynamic voltammograms. The values obtained are listed in Table 1.

Results

Hemoglobin Binding of Arylamines and Nitroarenes

Rats were dosed with arylamines or nitroarenes and sacrificed after 24 hr. Hemoglobin was hydrolyzed with NaOH. The released arylamine was extracted in hexane and analyzed by GC-MS. The results are summarized in Figures 1*A*,*B*, and 3. The following structure activity relationships were found:

The highest hemoglobin binding was obtained with compounds with a halogen in *para* position. A chlorine atom in *ortho* position reduces the formation of hemoglobin adducts drastically (1000-fold, for 2CA compared to 4CA). An additional *ortho* chlorine atom, as in 2,6-dichloroaniline (26DCA) or 2,3,4,5,6-pentachloroaniline (PCA), abolishes hemoglobin binding totally. All alkyl substituted amines have lower hemoglobin binding index (HBI) [(mmole compound/mole Hb)/(mmole compound/kg body weight) than aniline. The HBI of 3-ethylaniline (3EA) is higher than that of 2EA or 4EA. This might be explained by the fact that the oxidation of alkyl groups in *ortho* or *para* position to an amino group is facilitated compared with that of alkyl groups in *meta* position. Two methyl groups in *ortho* position, as in 2,6-dimethylaniline (26DMA) or 2,4,6-trimethylaniline (246TMA), almost abolish hemoglobin binding.

In general, lower hydrolyzable hemoglobin-adduct levels were found in rats that were given nitroarenes than in rats that were dosed with an equimolar amount of the corresponding arylamines [except for nitrobenzene (NB) and 4-flouronitrobenzene (4FNB)]. The SAR of nitroarenes and arylamines are similar (Figure 2). Highest hemoglobin binding was found for 4-chloronitrobenzene (4CNB) or 4-bromonitrobenzene (4BrNB). The least binding

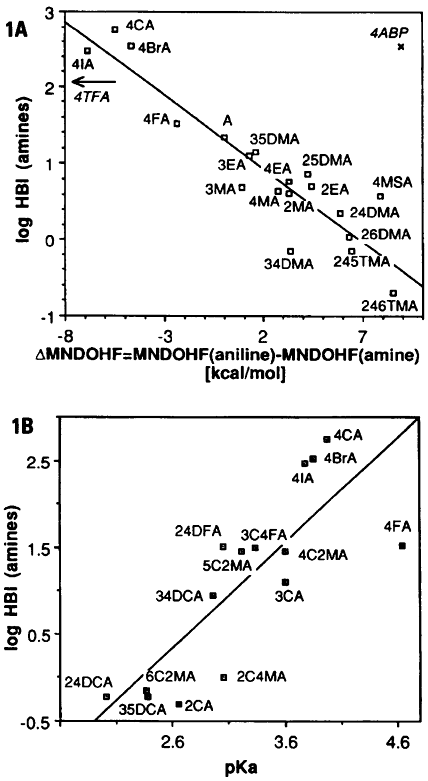

Figure 1. (*A*) Hemoglobin binding of arylamines in rats. The logarithm of the hemoglobin binding index (log HBI) was plotted against the relative stability of the corresponding nitrenium ion. Except for 4-aminobiphenyl (4ABP) and 4-(trifluoromethyl)-aniline (4TFA), all *para*- and alkyl-substituted arylamines (19 compounds) have been included in the regression analysis: log HBI = 1.31–0.194 ΔMNDOHF, $r = -0.92$. The abbreviations for the compounds are mentioned in the text, except for 4-bromoaniline (4BrA), 4-iodoaniline (4IA), 2,5-dimethylaniline (25DMA), 2,6-dimethylaniline (26DMA), 3,4-dimethylaniline (34DMA), and 3,5-dimethylaniline (35DMA). (*B*) Hemoglobin binding of arylamines in rats. The log HBI of aromatic amines with a halogen as a substituent was plotted against the pK_a: log HBI = –2.82 + 1.21 pK_a, $r = 0.81$.

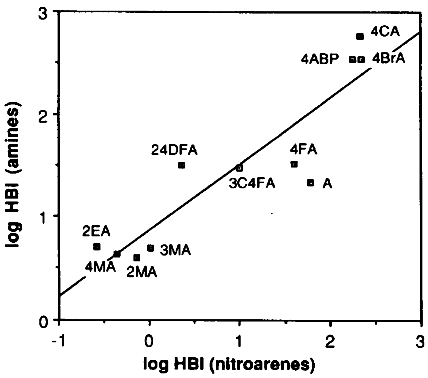

Figure 2. Hemoglobin binding of arylamines and nitroarenes in rats. The logarithm of the hemoglobin binding index (log HBI) of the arylamines was plotted against the log HBI of the corresponding nitroarenes: log HBI (amines) = 0.86 + 0.65 log HBI (nitroarenes), $r = 0.91$. The abbreviations used in the figure are for the arylamines.

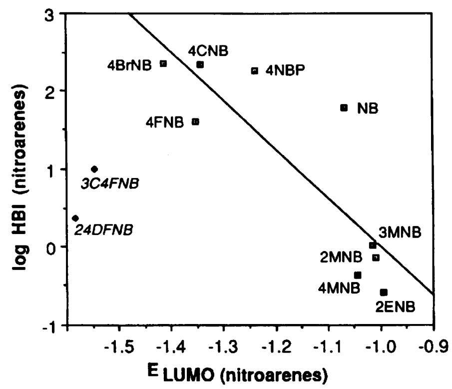

Figure 3. Hemoglobin binding of nitroarenes in rats. The logarithm of the hemoglobin binding index (log HBI) was plotted against the energy levels of the lowest unoccupied molecular orbital (E_{LUMO}) of the nitroarenes, calculated by the semiempirical method AM1. All *para*- and alkyl-substituted nitrobenzenes fit onto the regression curve: log HBI = –6.26–6.26 E_{LUMO}, $r = -0.85$. 3-Chloro-4-fluoronitrobenzene (3C4FNB) and 2,4-difluoronitrobenzene (24DFNB) are outliers. The HBI values published previously for nitrobenzene (NB) and 4-nitrobiphenyl (4NBP) were 79 *(40)* and 30 *(41)*, respectively. The abbreviations for the compounds are mentioned in the text, except for 2-methylnitrobenzene (2MNB) and 3-methylnitrobenzene (3MNB).

was found with nitrobenzenes with electron donating substituents (e.g., 4-methylnitrobenzene. 2,4-Dichloronitrobenzene (24DCNB) (*10*) and 2,3,4,5,6-pentachloronitrobenzene (PCNB) (*12*) do not bind to hemoglobin.

Correlation of Hemoglobin Binding with the Electronic Parameters for Arylamines and Nitroarenes

Arylamines. In order to bind to hemoglobin, arylamines first have to be oxidized to *N*-hydroxyarylamines. In the liver this process is mostly catalyzed by cytochrome P450. The product distribution of this oxidation process is described best with a nitrenium ion as an intermediate (*10,13*). In several studies, nitrenium ions have been postulated to be the ultimate carcinogens derived from arylamines (*14–20*). The electronic properties of the arylamines and of the corresponding nitrenium ions were calculated with the semiempirical programs MNDO, AM1, and PM3. All calculations were performed with the hydrogens of the amino group coplanar to the benzene ring, as the initial geometry of the arylamines. The initial geometry for the calculations of *ortho*-substituted methyl compounds is critical. Interestingly, equivalent starting geometries do not always yield the most stable conformation for all three programs. The most stable structures are obtained when the dihedral angle of C1–C2–CH2–H is 60° for MNDO and AM1 calculations. However, for PM3 calculations the most stable conformation was obtained starting with a dihedral angle of 0°. The energy differences between the two conformations are up to 1.7 kcal/mole for AM1 and MNDO, and 0.5 kcal/mole for PM3 calculations. The most stable geometry obtained for 2EA, 3EA, and 4EA is with the second carbon of the ethyl group out of the benzene ring plane with a dihedral angle C1-C2–CH2–CH3 of 90°. This change increases the stability of the conformer by approximately 2 kcal/mole for MNDO calculations and up to 1.1 kcal/mole for AM1 and PM3 calculations. However, the most stable structure for the nitrenium ion of 2EA has a dihedral angle C1–C2–CH2–CH3 of 0°. Care must be taken with the initial geometry of 4-methylmercaptoaniline (4MSA) for MNDO calculations. A dihedral angle C3–C4–S–CH3 of 90° increases the stability of this amine by 1.8 kcal/mole. The stability of the two possible rotamers of unsymmetrically substituted nitrenium ions were compared. In the previous publication (*10*) only the anti-conformer with the single proton on the nitrogen on the less substituted side was studied; H–N–C1–C2 = 180°. This was found not to represent the most stable conformer in all cases. Therefore, for the present study we have calculated stability of the syn conformer, with the proton on the nitrogen on the more substituted side with a dihedral angle H–N–C1–C2 = 0°. We found that compounds with ethyl or methyl groups in *ortho* position the anti conformers are up to 0.9 kcal/mole more stable. Nitrenium ions with an *ortho* chloro group are more stable in the syn conformation as calculated by MNDO and AM1. However, with PM3 calculations the anti conformation is up to 3.6 kcal/mole more stable. Ths syn conformation is more stable for most *meta*-substituted compounds. The values of the most stable conformers have been summarized in Table 1. The enthalpy change ΔMNDOHF of the isodesmic reaction (equation 1) yields a value for the stability of the nitrenium ions relative to that of aniline:ΔMNDOHF = MNDOHF(aniline)–MNDOHF(amine) =[Hf(nitrenium ion of aniline)–Hf(aniline)]–[Hf(nitrenium ion of amine)–Hf(amine)]; Hf=heat of formation calcu-

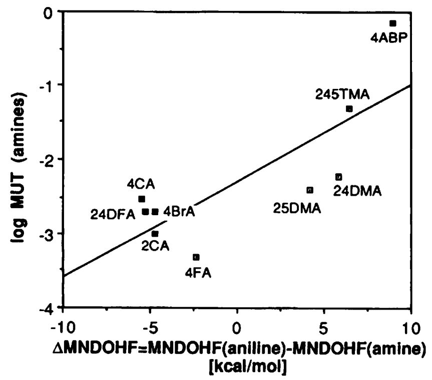

Figure 4. Mutagenicity of arylamines in *Salmonella typhimurium* TA98. The logarithm of mutagenicity [revertants per nmole compound] of arylamines was plotted against the relative stability of the nitrenium ions: log of mutagenicity, or log MUT = –2.29 + 0.13 ΔMNDOHF, r = 0.79.

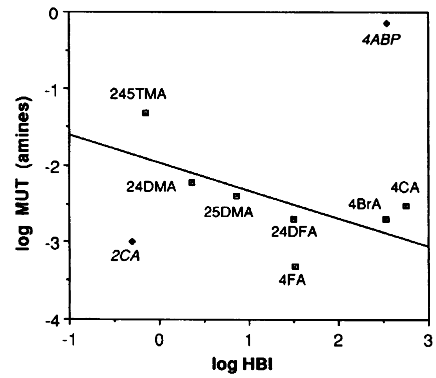

Figure 5. Mutagenicity of arylamines in *Salmonella typhimurium* TA98 and hemoglobin binding of arylamines in rats. 4-Aminobiphenyl (4ABP) and 2-chloroaniline (2CA) are not included in the regression analysis: r = –0.65. HBI, hemoglobin binding index; log MUT, logarithm of mutagenicity.

alkyl-substituted arylamines fit the regression curve very well. As in the case of arylamines, compounds with halogens in *ortho* or *meta* position are outliers. Analysis of a larger group of nitroarenes has been recently published (*9*).

Except for two arylamines (2,6-dichloroaniline [26DCA] and PCA) and two nitroarenes (24DCNB and PCNB), all arylamines and nitroarenes given to female Wistar rats formed hydrolyzable hemoglobin adducts. Therefore, for most compounds the potentially genotoxic intermediate—*N*-hydroxyarylamine—is bioavailable. Hemoglobin binding can be predicted with the electronic properties of the arylamines and nitroarenes. In a further analysis we determined if the same electronic properties are predictive for the carcinogenic, mutagenic, and cytotoxic potency of these arylamines and nitroarenes.

lated by MNDO. For amines in which nitrenium ions are less stable than the one of aniline, the enthalpy change is <0 kcal/mole.

The half wave oxidation potentials of arylamines correlate inversely with the stability of their nitrenium ions, calculated by MNDO, AM1, and PM3 with r = –0.93, –0.95, and 0.77, respectively. Arylamines that form a stable nitrenium ion have a smaller oxidation potential than arylamines that form more unstable nitrenium ions (e.g., 246TMA compared to 4CA). The oxidizability of the arylamines is directly proportional to the stability (ΔMNDOHF) of the corresponding nitrenium ions.

$$X\text{-}Ph\text{-}NH^+ + PhNH_2 \rightarrow X\text{-}Ph\text{-}NH_2 + PhNH^+ \quad [1]$$

The log HBI of all arylamines was plotted against ΔMNDOHF, ΔAM1HF, and ΔPM3HF. The best correlation (r = –0.92) was found for hemoglobin binding of *para*-substituted and alkyl-substituted arylamines and the stability of their nitrenium ions calculated with MNDO (Figure 1*A*). Poorer calculations are found when log HBI was plotted against the stability of nitrenium ions calculated by AM1, PM3, and the half wave oxidation potential with r = –0.80, –0.70, and 0.78, respectively. For AM1 and the half wave oxidation potential the correlations to log HBI increase to 0.91 and 0.86 if 4MSA is excluded. The compounds with halogens in *ortho* or *meta* postion, 3-(trifluoromethyl)-aniline (3TFA), 3-cyanoaniline (3CNA), 4-(trifluoromethyl)-aniline (4TFA), and 4ABP do not fit the curve. From these SARs found in rats, it appears that the pharmacokinetics or the metabolism of 4ABP and 4TFA is different from that of the other *para*- or alkyl-substituted arylamines. This difference also might be the case in humans. Bryant et al. (*21*) determined the levels of hemoglobin adducts of several monocyclic and bicyclic arylamines in smokers and nonsmokers. The amount of hemoglobin adducts of arylamines correlates positively with the smoker status only for three compounds (4-ABP, 3-aminobiphenyl, and 2-naphthylamine). The levels of hemoglobin adducts of the other arylamines were not related to smoking habits, although these amines are present in large amounts in cigarette smoke.

Hemoglobin binding of halogen-substituted arylamines can be predicted from the pK_a values (Figure 1*B*). The pK_a values were taken from the literature (*22,23*) except for the pK_a of 2,4-difluoroaniline (24DFA), 3-chloro-4-fluoroaniline (3C4FA), 2-chloro-4-methylaniline (2C4MA), 4-chloro-2-methylaniline (4C2MA), 5-chloro-2-methylaniline (5C2MA), and 6-chloro-2-methylaniline (6C2MA), which were estimated, according to Perrin et al. (*24*).

Nitroarenes. Nitroarenes have to be reduced to nitrosoarenes or to *N*-hydroxyarylamine to yield the same sulfinamide adducts as do arylamines. Therefore, hemoglobin binding of nitroarenes should depend on the ease of reduction of the nitro group. The energy level of the lowest unoccupied molecular orbital (E_{LUMO}) is a good parameter for predicting the reducibility of nitroarenes (*25,26*). Thirteen nitroarenes were tested for hemoglobin binding. The logarithm of the hemoglobin binding index was plotted against E_{LUMO} (Figure 3). The *para*- and

Mutagenicity of Arylamines and Nitroarenes

Mutagenicity of Arylamines. Are the electronic properties responsible for high HBI values the same as those responsible for high mutagenic and carcinogenic potency? For several arylamines, it has been shown that the mutagenic potency increases with the stability of the corresponding nitrenium ions (*14,15,27*). However, several compounds that are not mutagenic should be mutagenic according to their electronic properties, for example, aniline, 3CA, 2C4MA, and 4C2MA in *Salmonella typhimurium* TA98. Even with the inclusion of additional parameters (partition coefficients, the energy levels of the LUMO, and highest occupied molecular orbital of the arylamines) in a predictive equation, the mutagenicity of several arylamines is not predicted correctly (*28*).

In the present work, the data available for the mutagenic potency (*28–32*) of arylamines, expressed as logarithm of revertants per nmole compound (log MUT), were plotted against log HBI of the arylamines and the stability of the corresponding nitrenium ions. The mutagenic potency is directly proportional to the oxidizability of the arylamines (Figure 4) (e.g., 2,4,5-trimethylaniline [245TMA] is more mutagenic than 4CA), but inversely proportional to the amount of hemoglobin binding in rats (Figure 5). In addition, several arylamines (e.g., aniline, 3-chloroaniline [3CA], 2C4MA, and 4C2MA) that are not mutagenic, bind to hemoglobin.

Mutagenicity of Nitrobenzenes. For a set of about 20 nitroarenes (most of them were polyaromatic compounds) Klopman

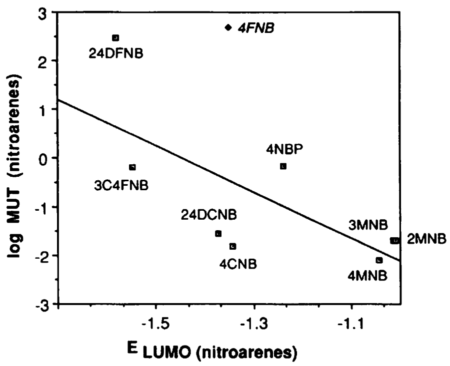

Figure 6. Mutagenicity of nitrobenzenes in *Salmonella typhimurium* TA100 (*34,36*) (less data points were available for the strain TA98 (*33*). The logarithm of mutagenicity (log MUT) was plotted against the calculated energy levels of the lowest unoccupied molecular orbital (E_{LUMO}) of the nitroarenes: $r = -0.72$ 4-Fluoronitrobenzene (4FNB) is not included in the regression analysis.

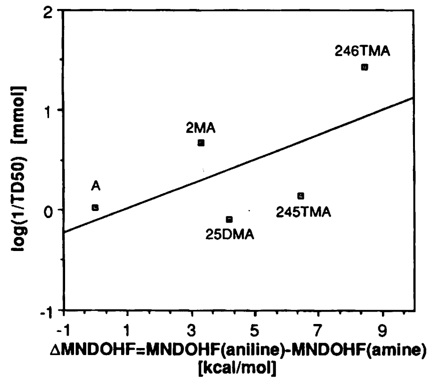

Figure 7. Carcinogenicity of arylamines in rats. Log $1/TD_{50}$ [mmole] was plotted against the relative stability of the nitrenium ions (ΔMNDOHF): $r = 0.63$. The TD_{50} values were obtained from Gold et al. (*38*).

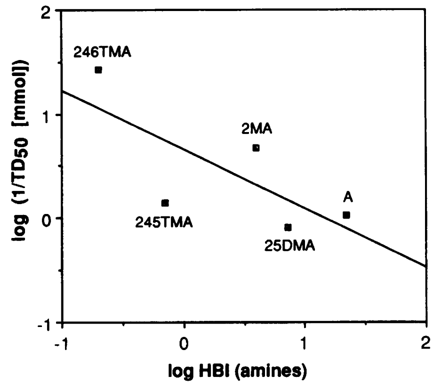

Figure 8. Carcinogenicity and hemoglobin binding of arylamines in rats. Log $1/TD_{50}$ [mmole] was plotted against log hemoglobin binding index (log HBI): $r = -0.74$. The TD_{50} values were obtained from Gold et al. (*38*).

et al. (*25*) and Maynard et al. (*26*) found a simple relationship between the electronic properties of nitroarenes and their mutagenicity. The mutagenicity increases with the ease of reduction of the nitroarenes. With the collection of further data, the models for predicting mutagenicity had to be modified. Additional factors describing the molecular dimensions, the degree of aromaticity, the hydrophobicity (*33,34* and literature cited therein), or the orientation of the nitro substituents (*35* and literature cited therein) were included to improve the predictive value of the equations.

The logarithm of the mutagenicity (*34,36*) of the nitroarenes which had been tested for homoglobin binding were plotted against the reducibility of the nitro group (E_{LUMO}). The mutagenic potency and the E_{LUMO} fit on a linear regression line (Figure 6). The mutagenicity of nitroarenes increases with the reducibility of the nitro group. Except for NB all compounds tested which bind to hemoblobin are mutagenic. Conversely, 24DCNB is mutagenic but does not bind to hemoglobin. Although hemoglobin binding increases with the reducibility of the nitro group, the correlation of mutagenicity with hemoglobin binding is very poor. This may be a function of insufficient data points, thus further analyses are necessary.

Carcinogenicity of Arylamines and Nitroarenes

Since the late 1970s, there has been a great deal of interest to elucidate the chemical properties of aromatic amines and nitroarenes that are responsible for the genotoxicity of this class of compounds (*16–18,37*). Much emphasis has been put on the structural features of the ultimate carcinogen. Although a bimolecular mechanism has been postulated in certain cases, most authors interpreted the carcinogenic potencies or the reactions with DNA with a nitrenium ion as an intermediate (*16–18,37*). For a comparison of hemoglobin binding with the carcinogenicity of arylamines in rats, the TD_{50} data compiled by Gold et al. (*38*) were used. TD_{50}-values (mmole) of arylamines tested in rats were found for only five monocyclic arylamines. Carcinogenicity increases with the oxidizability of the arylamines (Figure 7). Carcinogenicity is inversely proportional to hemoglobin binding for these compounds (Figure 8). Only in the case of the bifunctional arylamines 3,3′-dichlorobenzidine, 4,4′-methylenedianiline, 4,4′-methylenebis(2-chloroaniline), 4,4′-oxydianiline, and benzidine do carcinogenicity and hemoglobin binding correlate positively ($\log(1/TD_{50}$ [mmole]$) = 0.7 + 0.80 \log$ HBI; $r = 0.85$, data not shown). For the monocyclic nitroarenes investigated here, not enough data are available to study the correlation of carcinogenicity with hemoglobin binding.

Conclusions

Except for four compounds, it could be shown that after treating rats with nitroarenes or arylamines, hydrolyzable hemoglobin adducts are formed as a result of the formation of the potentially genotoxic intermediate *N*-hydroxyarylamine. Hemoglobin adducts are therefore a good dosimeter to biomonitor people exposed to a large array of arylamines and nitroarenes. The amount of hemoglobin binding decreases with the oxidizability of the arylamines, except for compounds that are substituted with halogens in *ortho* or *meta* position. For halogen-substituted arylamines, the amount of hemoglobin binding is directly proportional to the pK_a. The level of hemoglobin binding and mutagenicity is directly proportional to the reducibility of the nitroarenes, but hemoglobin binding and mutagenicity do not correlate. For arylamines, the electronic properties that are important for mutagenicity or carcinogenicity are not the same as those important for hemoglobin binding. Moreover, the correlation of carcinogenicity or mutagenicity of arylamines with the electronic properties of the corresponding nitrenium ions is not as good as that for hemoglobin binding. For an equation that better predicts carcinogenicity, other parameters that are important in the process of carcinogenesis may have to be included (e.g., cytotoxicity, K_m and V_{max} values of phase I and phase II enzymes, partition coefficient octanol-water).

Experiments to test for cytotoxic effects of metabolites of arylamines or nitroarenes have been performed with hepatocytes by O'Brien et al. (*5*) and de Silva et al. (*4*). For nitroarenes, it was shown that cytotoxicity is increased by electron-withdrawing groups in *para* position to the *nitro* group.

For arylamines, a new mechanism for cytotoxic effects of *N*-hydroxy-2-aminofluorene and *N*-hydroxy-2-aminophenanthrene has been proposed by Neumann et al. (*6,7*). *In vitro* experiments with mitochondria showed that *N*-hydroxy-2-aminofluorene or 2-nitrosofluorene caused cyanide-resistant oxygen consumption and calcium release. The formation of superoxide anion radical was demonstrated. However, several monocyclic arylamines (nitrosobenzene, 2-nitrosotoluene, 4-nitrosotoluene, *N,N*-dimethyl-4-nitrosotoluene, and 4-nitrosophenol) did not cause any oxidative stress; some of them (nitrosobenzene and 4-nitrosotoluene) induced calcium release. Further work has to be performed to see which structural parameters determine the cytotoxic properties of arylamines and their metabolites.

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